experiment showed that in both selections one type of Fab antibody (fingerprinting data not shown) was isolated. Fab1 was selected from the Hp-Hb complex-selections and Fab18 from the CD163 selection.

Screening of the selected anti-Hp-Hb complex and anti-CD163 repertoires- To identify Hp-Hb complex- and CD163 binding Fab antibody phage, an ELISA was performed in which Hp-Hb complexes or CD163 were coated and approximately 10^{10} phage expressed by single colonies were incubated. Bound phage were subsequently detected using an anti-M13 phage conjugate. The procedure was performed as described (Horn, I. R., Moestrup, S. K., van den Berg, B. M., Pannekoek, H., Nielsen, M. S., and van Zonneveld, A. J. (1995) J Biol Chem 270(20), 11770-5.). The number of unique Fabs was determined by PCR fingerprinting with two different fine-cutting restriction enzymes (Marks, J. D., Hoogenboom, H. R., Bonnert, T. P., McCafferty, J., Griffiths, A. D., and Winter, G. (1991) J Mol Biol 222(3), 581-97.). The results of the binding of Fab1-phage to these antigens are shown in Fig. 2*A*. As can be concluded from the figure, Fab1-phage strongly reacts with the Hp-Hb complex, whereas low binding to Hb and Hp is measured. Binding of Fab2-phage could not be detected to any of the antigens, indicating that the phage itself does not aspecifically interacts with any of the antigens (not shown). The observed differences can neither be accounted for by different coating efficiencies, since in a control experiment polyclonal sera against the different antigens react with the uncomplexed and the complexed proteins to the same extent (data not shown).

Preparation of Soluble Fabs and SPR analysis- The pCOMB3X vector allows for expression of soluble Fab by changing bacterial strains because of the presence of an amber codon in between the heavy chain first constant domain and the sequence encoding the M13 gene III product (13. Andris-Widhopf, J., Rader, C., Steinberger, P., Fuller, R., and Barbas, C. F., 3rd. (2000) J Immunol Methods 242(1-2), 159-81.). We have used the non-suppressor *E.coli* strain HB2151, which was kindly supplied by dr. P. Kristensen (department of Molecular Biology, University of Aarhus). Anti-Hp-Hb complex antibody Fab1 was purified from the bacterial supernatant upon overnight expression in super broth medium containing 1 mM isopropyl-β-D-thiogalactopyranoside. The anti-CD163 antibody Fab18 was purified from the bacterial cells after sonication in phenyl-methyl-sulfonyl fluoride-containing Tris-buffered saline. Both antibodies were purified to homogeneity after filtration in a single step affinity chromatography method using an anti-mouse kappa light chain sepharose-coupled antibody from Zymed Laboratories (AH Diagnostics, Denmark). Preparations were concentrated on Amicon concentrators and amounts were determined using the bicinchoninic acid method from Pierce. Purity was checked by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in combination with silverstaining. Fab activity was determined in an ELISA using an anti-HA-biotin conjugate (Hoffman-La Roche).

SPR analyses were performed in a BIAcore™2000 instrument (BIAcore AB, Sweden) as described (1,16). CM5 sensorchips were immobilized with approximately 55-66 fmoles per mm^2 of CD163, Hp, Hb or Hp-Hb complex. As a running buffer we used 10 mM HEPES-buffer containing 150 mM and 0.5 mM $CaCl_2$ at pH7.4. The data were plotted and subsequently fitted using the BIAevaluation 3.0 software. To further establish the binding characteristics of the isolated Fab phage. This procedure yielded approximately 0.5 mg pure Fab per liter of bacterial culture. The purity of Fabs has been determined by a silverstained polyacrylamide gel. Exact amounts of recombinant proteins were determined by applying the bicinchoninic acid method. After reassessing the binding activity of the pure Fab antibodies by ELISA, the binding of Fab1 to Hp-Hb complexes was further investigated with surface plasmon resonance. Using a sensorchip immobilized with both Hb, Hp and Hp-Hb complexes which allows for kinetic measurements, we derived a K_D constant of 3.9 nM for binding of Fab1 to Hp-Hb complexes. No binding to the other antigens could be detected at all, thereby demonstrating the complex-specificity of Fab1. These results are in line with the (phage) ELISA data. The binding curves are depicted in Fig. 2*B*. Anti-CD163 Fab18 demonstrates a low affinity for CD163 which is in the micromolar range (not shown).

CD163- ^{125}I-Hp-Hb complex-binding assays- Assays for measuring ^{125}Iodine-labeled Hp-Hb complex-binding to CD163 in the presence or absence of competing antibodies were performed essentially as described ((Kristiansen, M., Graversen, J. H., Jacobsen, C., Sonne, O., Hoffman, H. J., Law, S. K., and Moestrup, S. K. (2001) *Nature* **409**(6817), 198-201.) (Birn, H., Verroust, P. J., Nexo, E., Hager, H., Jacobsen, C., Christensen, E. I., and Moestrup, S. K. (1997) *J Biol Chem* **272**(42), 26497-504.)) Optimal coating conditions were first determined by using serial receptor dilutions followed by incubation with Hp-Hb complexes [(1:1) and (2:2) types)], labeled with ^{125}Iodine using the chloramine-T method. Binding assays were done using approximately 3000 counts per minute/well. Radioactivity was counted using a Packard gamma counter.

Cellular uptake and degradation experiments using ^{125}Iodine-labeled Hp-Hb complexes- Internalization and subsequent degradation in COS1 cells were described previously (Kozyraki, R., Fyfe, J., Kristiansen, M., Gerdes, C., Jacobsen, C., Cui, S., Christensen, E. I., Aminoff, M., de la Chapelle, A., Krahe, R., Verroust, P. J., and Moestrup, S. K. (1999) Nat Med 5(6), 656-61.). In brief, confluent cells were treated with 3000 counts per minute of ^{125}I-labeled Hp-Hb complexes and incubated concommitantly with a range of Fab antibody concentrations up to micromolar amounts. Supernatant was counted each 30 minutes to assess the degradation rate and after 4 hours cells were stringently washed followed by counting of internalized radioactivity. As can be seen in Fig. 13, already at nanomolar concentrations a 50% inhibition of binding is measured. The anti-CD163 Fab18 antibody also inhibits the

binding, albeit at micromolar concentrations. In the presence of micromolar amounts of an irrelevant Fab antibody (FabA8, (Horn, I. R., Moestrup, S. K., van den Berg, B. M., Pannekoek, H., Nielsen, M. S., and van Zonneveld, A. J. (1995) J Biol Chem 270(20), 11770-5.)) at least 80% tracer is still bound. The data were obtained using the (2:2) Hp form, however, in a set of experiments using the (1:1) form similar results were obtained, consistent with the competition data described previously (1. Kristiansen, M., Graversen, J. H., Jacobsen, C., Sonne, O., Hoffman, H. J., Law, S. K., and Moestrup, S. K. (2001) Nature 409(6817), 198-201.). Using ELISA and SPR methods, we were also able to demonstrate the inhibition of Hp-Hb complex binding to CD163 by Fab1 (data not shown).